

WHAT IS CLAIMED IS:

- 1 1. An isolated infectious chimeric respiratory syncytial virus (RSV)
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large
3 polymerase protein (L), a RNA polymerase elongation factor, and a partial or complete
4 RSV background genome or antigenome of a human or bovine RSV combined with one
5 or more heterologous gene(s) and/or genome segment(s) of a different RSV to form a
6 human-bovine chimeric RSV genome or antigenome.
- 1 2. The chimeric RSV of claim 1, wherein said one or more
2 heterologous gene(s) and/or genome segment(s) include one or more RSV NS1, NS2, N,
3 P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene(s) or genome segment(s) or a leader,
4 trailer or intergenic region of the RSV genome or a segment thereof.
- 1 3. The chimeric RSV of claim 2, wherein said one or more
2 heterologous gene(s) and/or genome segment(s) includes one or more gene(s) or genome
3 segment(s) encoding a RSV F, G and/or SH glycoprotein or an immunogenic domain or
4 epitope thereof.
- 1 4. The chimeric RSV of claim 1, wherein the human-bovine chimeric
2 RSV genome or antigenome encodes a chimeric glycoprotein having both human and
3 bovine glycoprotein domains or immunogenic epitopes.
- 1 5. The chimeric RSV of claim 4, wherein said one or more
2 heterologous gene(s) and/or genome segment(s) includes a gene segment encoding a
3 glycoprotein ectodomain.
- 1 6. The chimeric RSV of claim 1, wherein a heterologous gene or
2 genome segment is substituted for a counterpart gene or genome segment in a partial RSV
3 background genome or antigenome.
- 1 7. The chimeric RSV of claim 1, wherein a heterologous gene or
2 genome segment is added adjacent to, within, or as a replacement to, a noncoding region
3 of the partial or complete RSV background genome or antigenome.

1 8. The chimeric RSV of claim 1, wherein a heterologous gene or
2 genome segment is added or substituted at a position corresponding to a wild-type gene
3 order position of a counterpart gene or genome segment within the partial or complete
4 RSV background genome or antigenome.

1 9. The chimeric RSV of claim 1, wherein a heterologous gene or
2 genome segment is added or substituted at a position that is more promoter-proximal or
3 promoter-distal compared to a wild-type gene order position of a counterpart gene or
4 genome segment within the partial or complete RSV background genome or antigenome.

1 10. The chimeric RSV of claim 1, wherein the chimeric genome or
2 antigenome comprises a partial or complete human RSV background genome or
3 antigenome combined with one or more heterologous gene(s) and/or genome segment(s)
4 from a bovine RSV.

1 11. The chimeric RSV of claim 10, wherein one or more genes selected
2 from N, P, NS1, NS2, M2-1 and M of a human RSV is/are replaced by one or more
3 heterologous gene(s) from a bovine RSV.

1 12. The chimeric RSV of claim 11, wherein both N and P genes of a
2 human RSV are replaced by counterpart N and P genes from a bovine RSV.

1 13. The chimeric RSV of claim 11, wherein both NS1 and NS2 genes
2 of a human RSV are replaced by counterpart NS1 and NS2 genes from a bovine RSV.

1 14. The chimeric RSV of claim 11, wherein two or more of the M2-1,
2 M2-2 and L genes are replaced by counterpart genes from a bovine RSV

1 15. The chimeric RSV of claim 11, wherein each of the N, P, NS1,
2 NS2, M2-1 and M genes of a human RSV are replaced by counterpart N, P, NS1, NS2,
3 M2-1 and M genes from a bovine RSV.

1 16. The chimeric RSV of claim 1, wherein the chimeric genome or
2 antigenome comprises a partial or complete bovine RSV background genome or
3 antigenome combined with one or more heterologous gene(s) and/or genome segment(s)
4 from a human RSV.

1 17. The chimeric RSV of claim 16, wherein one or more human RSV
2 glycoprotein genes selected from F, G and SH, or one or more genome segment(s)
3 encoding cytoplasmic domain, transmembrane domain, ectodomain or immunogenic
4 epitope portion(s) of F, G, and/or SH is/are added or substituted within a partial or
5 complete bovine RSV background genome or antigenome.

1 18. The chimeric RSV of claim 17, wherein one or both human RSV
2 glycoprotein genes F and G is/are substituted to replace one or both counterpart F and G
3 glycoprotein genes in a partial bovine RSV background genome or antigenome.

1 19. The chimeric RSV of claim 17, wherein the human-bovine
2 chimeric genome or antigenome incorporates antigenic determinants from one or both
3 subgroup A and subgroup B human RSV.

1 20. The chimeric RSV of claim 17, wherein both human RSV
2 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein
3 genes in the bovine RSV background genome or antigenome.

1 21. The chimeric RSV of claim 20, which is rBRSV/A2.

1 22. The chimeric RSV of claim 9, wherein one or more human RSV
2 glycoprotein genes selected from F, G and SH is/are added or substituted at a position that
3 is more promoter-proximal compared to a wild-type gene order position of a counterpart
4 gene or genome segment within a partial or complete bovine RSV background genome or
5 antigenome.

1 23. The chimeric RSV of claim 22, wherein both human RSV
2 glycoprotein genes G and F are substituted at gene order positions 1 and 2, respectively,
3 to replace counterpart G and F glycoprotein genes deleted at wild type positions 7 and 8,
4 respectively in a partial bovine RSV background genome or antigenome.

1 24. The chimeric RSV of claim 23, which is rBRSV/A2-G1F2

1 25. The chimeric RSV of claim 17, wherein the chimeric genome or
2 antigenome is further modified by addition or substitution of one or more additional
3 heterologous gene(s) or genome segment(s) from a human RSV within the partial or

4 complete bovine background genome or antigenome to increase genetic stability or alter
5 attenuation, reactogenicity or growth in culture of the chimeric virus.

1 26. The chimeric RSV of claim 16, wherein one or more human RSV
2 envelope-associated genes selected from F, G, SH, and M is/are added or substituted
3 within a partial or complete bovine RSV background genome or antigenome.

1 27. The chimeric RSV of claim 26, wherein one or more human RSV
2 envelope-associated genes selected from F, G, SH, and M is/are added or substituted
3 within a partial bovine RSV background genome or antigenome in which one or more
4 envelope-associated genes selected from F, G, SH, and M is/are deleted.

1 28. The chimeric RSV of claim 27, wherein human RSV envelope-
2 associated genes F, G, and M are added within a partial bovine RSV background genome
3 or antigenome in which envelope-associated genes F, G, SH, and M are deleted.

1 29. The chimeric RSV of claim 28, which is rBRSV/A2-MGF.

1 30. The chimeric RSV of claim 1, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 present within a panel of mutant human RSV strains, said panel comprising cpts RSV 248
4 (ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV 248/955 (ATCC VR
5 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC VR 2451), cpts RSV
6 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542), and RSV B-1 cp-23
7 (ATCC VR 2579).

1 31. The chimeric RSV of claim 30, wherein the chimeric genome or
2 antigenome incorporates attenuating mutations adopted from different mutant RSV
3 strains.

1 32. The chimeric RSV of claim 1, wherein the chimeric genome or
2 antigenome incorporates at least one and up to a full complement of attenuating mutations
3 specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or
4 Thr523 in the RSV F gene, Asn43, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or
5 His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start
6 sequence of gene M2.

1 33. The chimeric RSV of claim 32, wherein the chimeric genome or
2 antigenome incorporates at least two attenuating mutations.

1 34. The chimeric RSV of claim 32, wherein the chimeric genome or
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide
3 changes in a codon specifying the mutation.

1 35. The chimeric RSV of claim 1, wherein the chimeric genome or
2 antigenome further comprises a nucleotide modification specifying a phenotypic change
3 selected from a change in growth characteristics, attenuation, temperature-sensitivity,
4 cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1 36. The chimeric RSV of claim 35, wherein the nucleotide
2 modification alters a SH, NS1, NS2, M2ORF2, or G gene of the chimeric virus.

1 37. The chimeric RSV of claim 36, wherein a SH, NS1, NS2, M2
2 ORF2, or G gene of the chimeric virus is deleted in whole or in part or expression of the
3 gene is ablated by introduction of one or more stop codons in an open reading frame of
4 the gene.

1 38. The chimeric RSV of claim 35, wherein the nucleotide
2 modification comprises a nucleotide deletion, insertion, substitution, addition or
3 rearrangement of a cis-acting regulatory sequence of a selected gene within the chimeric
4 RSV genome or antigenome.

1 39. The chimeric RSV of claim 38, wherein a gene end (GE) signal of
2 the NS1 or NS2 gene is modified.

1 40. The chimeric RSV of claim 35, wherein the nucleotide
2 modification comprises an insertion, deletion, substitution, or rearrangement of a
3 translational start site within the chimeric genome or antigenome.

1 41. The chimeric RSV of claim 40, wherein the translational start site
2 for a secreted form of the RSV G glycoprotein is ablated.

1 42. The chimeric RSV of claim 35, wherein the chimeric genome or
2 antigenome is modified to encode a non-RSV molecule selected from a cytokine, a T-

3 helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of
4 eliciting a protective immune response in a mammalian host.

1 43. The chimeric RSV of claim 35, which incorporates one or more
2 gene(s) and/or genome segment(s) from parainfluenza virus (PIV).

1 44. The chimeric RSV of claim 43, wherein the chimeric genome or
2 antigenome encodes a PIV HN or F glycoprotein or immunogenic domain or epitope
3 thereof.

1 45. The chimeric RSV of claim 44, wherein the chimeric genome or
2 antigenome encodes an ectodomain or immunogenic epitope of HN or F of PIV1, PIV2,
3 or PIV3.

1 46. The chimeric RSV of claim 1 which is a virus.

1 47. The chimeric RSV of claim 1 which is a subviral particle.

1 48. A method for stimulating the immune system of an individual to
2 induce protection against RSV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric RSV of claim 1 combined with a
4 physiologically acceptable carrier.

1 49. The method of claim 48, wherein the chimeric RSV is administered
2 in a dose of 10^3 to 10^6 PFU.

1 50. The method of claim 48, wherein the chimeric RSV is administered
2 to the upper respiratory tract.

1 51. The method of claim 48, wherein the chimeric RSV is administered
2 by spray, droplet or aerosol.

1 52. The method of claim 48, wherein the chimeric RSV is administered
2 to an individual seronegative for antibodies to RSV or possessing transplacentally
3 acquired maternal antibodies to RSV.

1 53. The method of claim 48, wherein the chimeric RSV elicits an
2 immune response against either human RSV A or RSV B.

1 54. The method of claim 48, wherein the chimeric RSV elicits an
2 immune response against both human RSV A and RSV B.

1 55. The method of claim 48, wherein the chimeric RSV elicits an
2 immune response against either human RSV A or RSV B and is co-administered with an
3 immunologically sufficient amount of a second attenuated RSV capable of eliciting an
4 immune response against human RSV A or RSV B, whereby an immune response is
5 elicited against both human RSV A and RSV B.

1 56. The method of claim 55, wherein the chimeric RSV and second
2 attenuated RSV are administered simultaneously as a mixture.

1 57. An immunogenic composition to elicit an immune response against
2 RSV comprising an immunologically sufficient amount of the chimeric RSV of claim 1 in
3 a physiologically acceptable carrier.

1 58. The immunogenic composition of claim 57, formulated in a dose of
2 10^3 to 10^6 PFU.

1 59. The immunogenic composition of claim 57, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 60. The immunogenic composition of claim 57, wherein the chimeric
2 RSV elicits an immune response against either human RSV A or RSV B.

1 61. The immunogenic composition of claim 57, wherein the chimeric
2 RSV elicits an immune response against both human RSV A and RSV B

1 62. The immunogenic composition of claim 57, wherein the chimeric
2 RSV elicits an immune response against either human RSV A or RSV B and wherein the
3 composition further comprises an immunologically sufficient amount of a second
4 attenuated RSV capable of eliciting an immune response against human RSV A or RSV
5 B, whereby the composition elicits an immune response against both human RSV A and
6 RSV B.

1 63. An isolated polynucleotide molecule comprising a chimeric RSV
2 genome or antigenome which includes a partial or complete RSV background genome or

3 antigenome of a human or bovine RSV combined with one or more heterologous gene(s)
4 or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome
5 or antigenome.

1 64. The isolated polynucleotide of claim 63, wherein said one or more
2 heterologous gene(s) and/or genome segment(s) include one or more RSV NS1, NS2, N,
3 P, M, SH, M2(ORF1), M2(ORF2), L, F or G gene(s) or genome segment(s) or a leader,
4 trailer or intergenic region of the RSV genome or a segment thereof.

1 65. The isolated polynucleotide of claim 63, wherein a heterologous
2 gene or genome segment is substituted for a counterpart gene or genome segment in a
3 partial RSV background genome or antigenome.

1 66. The isolated polynucleotide of claim 63, wherein a heterologous
2 gene or genome segment is added adjacent to, within, or as a replacement to, a noncoding
3 region of the partial or complete RSV background genome or antigenome.

1 67. The isolated polynucleotide of claim 63, wherein a heterologous
2 gene or genome segment is added or substituted at a position that is more promoter-
3 proximal or promoter-distal compared to a wild-type gene order position of a counterpart
4 gene or genome segment within the partial or complete RSV background genome or
5 antigenome.

1 68. The isolated polynucleotide of claim 63, wherein the chimeric
2 genome or antigenome comprises a partial or complete human RSV background genome
3 or antigenome combined with one or more heterologous gene(s) and/or genome
4 segment(s) from a bovine RSV.

1 69. The isolated polynucleotide of claim 68, wherein one or more
2 genes selected from N, P, NS1, NS2, M2-1 and M of a human RSV is/are replaced by one
3 or more heterologous gene(s) from a bovine RSV.

1 70. The isolated polynucleotide of claim 68, wherein both N and P
2 genes of a human RSV are replaced by counterpart N and P genes from a bovine RSV.

1 71. The isolated polynucleotide of claim 68, wherein both NS1 and
2 NS2 genes of a human RSV are replaced by counterpart NS1 and NS2 genes from a
3 bovine RSV.

1 72. The isolated polynucleotide of claim 68, wherein two or more of
2 the M2-1, M2-2 and L genes are replaced by counterpart genes from a bovine RSV

1 73. The isolated polynucleotide of claim 63, wherein the chimeric
2 genome or antigenome comprises a partial or complete bovine RSV background genome
3 or antigenome combined with one or more heterologous gene(s) and/or genome
4 segment(s) from a human RSV.

1 74. The isolated polynucleotide of claim ~~73~~, wherein one or more
2 human RSV glycoprotein genes selected from F, G and SH, or one or more genome
3 segment(s) encoding cytoplasmic domain, transmembrane domain, ectodomain or
4 immunogenic epitope portion(s) of F, G, and/or SH is/are added or substituted within a
5 partial or complete bovine RSV background genome or antigenome.

1 75. The isolated polynucleotide of claim 74, wherein one or both
2 human RSV glycoprotein genes F and G is/are substituted to replace one or both
3 counterpart F and G glycoprotein genes in a partial bovine RSV background genome or
4 antigenome.

1 76. The isolated polynucleotide of claim 75, wherein both human RSV
2 glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein
3 genes in the bovine RSV background genome or antigenome.

1 77. The isolated polynucleotide of claim 67, wherein one or more
2 human RSV glycoprotein genes selected from F, G and SH is/are added or substituted at a
3 position that is more promoter-proximal compared to a wild-type gene order position of a
4 counterpart gene or genome segment within a partial or complete bovine RSV
5 background genome or antigenome.

1 78. The isolated polynucleotide of claim 77, wherein both human RSV
2 glycoprotein genes G and F are substituted at gene order positions 1 and 2, respectively,

3 to replace counterpart G and F glycoprotein genes deleted at wild type positions 7 and 8,
4 respectively in a partial bovine RSV background genome or antigenome.

1 79. The isolated polynucleotide of claim 73, wherein the chimeric
2 genome or antigenome is further modified by addition or substitution of one or more
3 additional heterologous gene(s) or genome segment(s) from a human RSV within the
4 partial or complete bovine background genome or antigenome to increase genetic stability
5 or alter attenuation, reactogenicity or growth in culture of the chimeric virus.

1 80. The isolated polynucleotide of claim 73, wherein one or more
2 human RSV envelope-associated genes selected from F, G, SH, and M is/are added or
3 substituted within a partial or complete bovine RSV background genome or antigenome.

1 81. The isolated polynucleotide of claim 80, wherein human RSV
2 envelope-associated genes F, G, and M are added within a ~~partial~~ bovine RSV
3 background genome or antigenome in which envelope-associated genes F, G, SH, and M
4 are deleted.

1 82. The isolated polynucleotide molecule of claim 63, wherein the
2 human-bovine chimeric genome or antigenome incorporates antigenic determinants from
3 both subgroup A and subgroup B human RSV.

1 83. The isolated polynucleotide molecule of claim 63, wherein the
2 chimeric genome or antigenome is further modified by incorporation of one or more
3 attenuating mutations.

1 84. The isolated polynucleotide molecule of claim 63, further
2 comprising a nucleotide modification specifying a phenotypic change selected from a
3 change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation,
4 plaque size, host-range restriction, or a change in immunogenicity.

1 85. The isolated polynucleotide molecule of claim 63, wherein a SH,
2 NS1, NS2, M2ORF2, or G gene is modified.

1 86. The isolated polynucleotide molecule of claim 85, wherein the SH,
2 NS1, NS2, M2 ORF2, or G gene is deleted in whole or in part or expression of the gene is
3 ablated by introduction of one or more stop codons in an open reading frame of the gene.

1 87. The isolated polynucleotide molecule of claim 59, wherein the
2 nucleotide modification comprises a nucleotide deletion, insertion, addition or
3 rearrangement of a cis-acting regulatory sequence of a selected RSV gene within the
4 chimeric RSV genome or antigenome.

1 88. A method for producing an infectious attenuated chimeric RSV
2 particle from one or more isolated polynucleotide molecules encoding said RSV,
3 comprising:

4 expressing in a cell or cell-free lysate an expression vector comprising an
5 isolated polynucleotide comprising a partial or complete RSV background genome or
6 antigenome of a human or bovine RSV combined with one or more heterologous gene(s)
7 or genome segment(s) of a different RSV to form a human-bovine chimeric RSV genome
8 or antigenome, and RSV N, P, L and RNA polymerase elongation factor proteins.

1 89. The method of claim 88, wherein the chimeric RSV genome or
2 antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed
3 by two or more different expression vectors.

1 90. The chimeric RSV of claim 1, wherein the bovine-human chimeric
2 genome or antigenome comprises a partial or complete RSV vector genome or
3 antigenome combined with one or more heterologous genes or genome segments
4 encoding one or more antigenic determinants of one or more heterologous pathogens.

1 91. The chimeric RSV of claim 90, wherein said one or more
2 heterologous pathogens is a heterologous RSV and said heterologous gene(s) or genome
3 segment(s) encode(s) one or more RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2),
4 L, F or G protein(s) or fragment(s) thereof.

1 92. The chimeric RSV of claim 90, wherein the vector genome or
2 antigenome is a partial or complete RSV A genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of a RSV B
4 subgroup virus.

1 93. The chimeric RSV of claim 90, wherein the chimeric genome or
2 antigenome incorporates one or more gene(s) or genome segment(s) of a BRSV that
3 specifies attenuation.

1 94. The chimeric RSV of claim 90, wherein one or more HPIV1,
2 HPIV2, or HPIV3 gene(s) or genome segment(s) encoding one or more HN and/or F
3 glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to
4 or incorporated within the partial or complete HRSV vector genome or antigenome.

1 95. The chimeric RSV of claim 90, wherein a transcription unit
2 comprising an open reading frame (ORF) of an HPIV2 HN or F gene is added to or
3 incorporated within the chimeric HRSV vector genome or antigenome.

1 96. The chimeric RSV of claim 35, wherein the vector genome or
2 antigenome is a partial or complete BRSV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 HRSV(s).

1 97. The chimeric RSV of claim 96, wherein the partial or complete
2 BRSV genome or antigenome incorporates one or more gene(s) or genome segment(s)
3 encoding one or more HRSV glycoprotein genes selected from F, G and SH, or one or
4 more genome segment(s) encoding cytoplasmic domain, transmembrane domain,
5 ectodomain or immunogenic epitope portion(s) of F, G, and/or SH of HRSV.

1 98. The chimeric RSV of claim 90, wherein the vector genome or
2 antigenome is a partial or complete HRSV or BRSV genome or antigenome and the
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza
7 viruses.

1 99. The chimeric RSV of claim 98, wherein said one or more
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F
3 proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins,

4 mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2
5 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus
6 gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein
7 Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and
8 NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes
9 thereof.

1 100. The chimeric RSV of claim 99, wherein the heterologous pathogen
2 is measles virus and the heterologous antigenic determinant(s) is/are selected from the
3 measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 101. The chimeric RSV of claim 100, wherein a transcription unit
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or
3 incorporated within a HRSV vector genome or antigenome.